

ON THE VALUE OF EXPERIMENTAL TUBERCULOSIS IN DIAGNOSIS.

By SHERIDAN DELEPINE, M.B.,

Professor of Pathology, Owens College, Manchester.

PRELIMINARY REMARKS.

THE importance of the inoculation method in the study of tuberculosis is recognised by all investigators. Putting aside the imperfect attempts of Klencke, it may be said that since 1865, when Villemin published the results of his most careful and convincing investigations, most of the observers who have studied tuberculosis have used his method.

Subcutaneous inoculations were at first supposed to be open to serious objections, to which allusion has been made by Koch in his celebrated paper on the Etiology of Tuberculosis,¹ where he speaks of "unnoticed errors of manipulation," and of "mistaking spontaneous tuberculosis for an artificially induced form of the disease." To remove these objections some previous observers had introduced modifications of Villemin's method. Thus Cohnheim and Solomonsen, in 1877, had used inoculation into the anterior chamber of the rabbit's eye; and Hippolyte Martin, in 1879, had insisted upon the importance of inoculations in series.

Nevertheless Villemin's method apparently became obsolete on the day when Koch was able to demonstrate the bacillus of tuberculosis in tuberculous products. Some observers, however, mostly of the French school, have pointed out that the bacillus tuberculosis was often so very difficult to discover in certain tuberculous products that the inoculation method had still many applications, this being not only true of surgical tuberculous products but also of those that come under the notice of the physician.²

Anybody who has had an opportunity to look for the tubercle bacillus in specimens in which bacilli were very scanty, knows how long and discouraging such a search is at times, and when after tedious efforts one finds nothing, and the symptoms point distinctly to a tuberculous process, one is not tempted to attach undue importance to the negative results of the microscopical examination.

If it could be proved that the inoculation method is free from the objections to which I have alluded briefly, one would be ready to admit that this method does present great advantages in doubtful cases, one of the chief advan-

¹ New Sydenham Society. *Microparasites in Disease. Selected Essays*, p. 69. 1884.

² See Arloing, *Congrès pour l'Etude de la Tuberculose*, 1888, p. 404. Leloir, *ibid.*, p. 412. Verneuil and Clado, *ibid.*, p. 415.

ages being that negative results may, if proper precautions be taken, be considered to be practically as reliable as positive results.

NATURE OF THIS INVESTIGATION.

It is my object here to show that Villemain's method of subcutaneous inoculation is capable of giving most definite and reliable results within a much shorter space of time than is generally supposed to be necessary. In this I agree with Verneuil and Clado.³

The remarks which I will now offer are based upon experiments, made in the course of various investigations, on 90 guinea-pigs, rabbits, rats, and mice, during the last year. Of these experiments some need not be further alluded to since they were made with non-tuberculous products and served only as control experiments. The experiments on other animals than guinea-pigs may also for the present be left out, as I wish specially to show the value of the guinea-pig as a living reagent for tuberculosis. I may say, however, that I consider the rabbit an unsuitable animal for work of this kind.

I inoculated 28 guinea-pigs with tuberculous matter from various sources. The place of inoculation which I have selected after a few trials was the inner aspect of the leg at about the level of the knee. I may say at once that subcutaneous inoculation at this spot gives, in practically the same time, far more typical results than inoculation into the peritoneal cavity. Arloing has come to the same conclusion.⁴

I will confine my remarks to the results obtained with tuberculous sputa, or with very small quantities of pure cultivations of human tubercle bacilli, but I have experimented also with other tuberculous products. The sputa were from different sources, some very rich, others very poor in tubercle bacilli. The cultivations used were very virulent; the bacillus had been cultivated outside the body for more than a year, and for about six months it had been cultivated on glycerine agar-agar, on which it grew with considerable difficulty. I will not give here a lengthy description of the results obtained after guinea-pigs have been inoculated; this has been done by many observers. I will only point out the order in which the most important lymphatic ganglia, as well as the spleen, liver, and lungs, become affected. The guinea-pigs being killed at various intervals after inoculation were dissected so as to display in each case the following parts:—

The popliteal ganglia on both sides, the superficial and deep inguinal ganglia on both sides, the sublumbar ganglia, the retrohepatic ganglion, the mediastinal and bronchial ganglia, the deep cervical ganglia, the subscapular ganglia, the spleen, liver, and lungs.

In all the cases some of the lesions were examined microscopically and tubercle bacilli found in them. Pure cultivations of tubercle bacilli were also obtained from the spleen and sublumbar ganglion in two cases.

RESULTS.

The results obtained with pure cultivations presented a remarkable uniformity; with sputa there were slight irregularities in the rate of extension, but the results obtained were quite as clear and definite as in the experiments made with pure cultivations. I will now sum up the results obtained in a few typical cases.

³ *Loc. cit.*

⁴ *Leçons sur la Tuberculose*, 1892, p. 112.

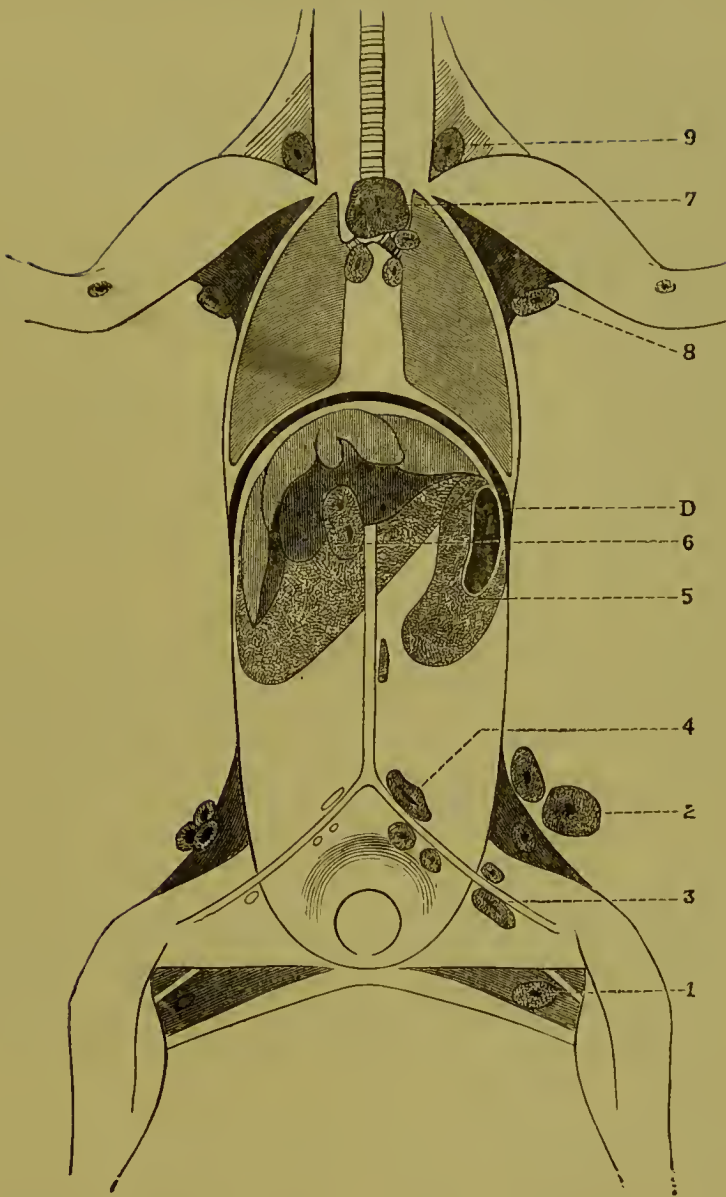


Diagram of a guinea-pig four or five weeks after subcutaneous inoculation of tuberculous matter on the inner aspect of the left leg. The black outline indicates approximately the normal size; the lighter outline the size of the organs when the tuberculous processes have become established. 1. Popliteal ganglion. 2. Superficial inguinal ganglion. 3. Deep inguinal ganglion. 4. Sub-lumbar ganglion. 5. Spleen. 6. Retrohepatic ganglion. 7. Mediastinal and peribronchial ganglion. 8. Subscapular ganglia. 9. Inferior deep cervical ganglia. D. Diaphragm.

Twelve days after inoculation on the left side with about $\frac{1}{2}$ of a small colony of bacilli (under 1 mm. in diameter), the following parts were found affected:—Left popliteal ganglion quite distinctly; the left superficial inguinal ganglia distinctly; the left sublumbar ganglion distinctly; the retrohepatic ganglion was slightly affected; the spleen slightly affected.

Twenty Days after Inoculation with a Pure Cultivation.—The above organs were much more enlarged, and several ganglia were already undergoing cheesy degeneration. In addition, the following organs were also distinctly affected—liver, mediastinal and bronchial ganglia.

(Twenty Days after Inoculation with Certain Specimens of Sputa.—The following parts were already distinctly affected:—Left popliteal ganglion, left superficial and deep inguinal ganglia, left sublumbar ganglia, the retrohepatic ganglia, the mediastinal and bronchial ganglia, the spleen, the liver. The lungs were much congested, and possibly tuberculosis was beginning to extend to them.)

Twenty-six Days after Inoculation with Pure Cultivation.—All the above organs were still more affected, and in addition there were distinct changes in the following: The subscapular ganglia, the inferior deep cervical ganglia; the lungs showed signs of becoming involved.

Thirty Days after Inoculation with Pure Cultivation.—The lungs were certainly affected. The right superficial inguinal ganglia were beginning to be involved.

(Forty-three Days after Inoculation with Sputum.—All the above organs were affected with the exception of the right inguinal ganglia.)

Fifty-two Days after Inoculation with Sputum.—The right inguinal ganglia were enlarged.

Sixty-two Days after Inoculation with a Pure Cultivation.—The same organs were affected as at the earlier dates, but the cheesy degeneration of tubercles, the enlargement and necrosis of the liver and spleen were very much more marked.

Seventy-four Days after Inoculation with a Pure Cultivation.—There was a slight indication of swelling of the right sublumbar ganglion; the right popliteal was still normal in appearance.

These results agree in the main with those given by Arloing, but there are, however, some differences.

According to Arloing the mode and rate of extension are as follows: *Fifteenth Day.*—Superficial and deep inguinal ganglia on the side of inoculation. *Twentieth Day.*—Sublumbar ganglia. *Twenty-second to Twenty-fifth Day.*—Spleen, retrohepatic ganglia. After this the bronchial ganglia and the lungs would become affected. *Sixtieth Day.*—The tuberculosis would be general.

It will be evident from this that I have found evidence of affection of the lymphatics on the same side of the body, and also of the liver, spleen, and lungs at much earlier dates than Arloing had, in fact more than a week earlier. On the other hand, I would not feel inclined to say that tuberculosis had, in my case, become general on the 60th day, nor even on the 74th or 97th or 106th day, since at those dates I have only found a few lymphatics of the lower part of the body on the side opposite to that inoculated showing any signs of tuberculosis.⁵

As I do not wish here to deal with any other point than the diagnostic value of inoculation, I will not discuss the facts which I have just brought forward, but I will summarise them as follows:

Products containing even a small number of tubercle bacilli will, when inoculated under the skin of the inner aspect of the leg of a guinea-pig at the level of the knee, cause tuberculosis of a certain number of organs in the following order:

During the Second Week.—The lymphatic ganglia on the same side of the body below the diaphragm and the spleen.

During the Third Week.—The liver, mediastinal, and bronchial ganglia.

During the Fourth Week.—The lungs, cervical and axillary ganglia.

⁵ It must be remembered that these differences may be due to external circumstances or to the breed of animals used. I do not wish, however, to do more than indicate this slight discrepancy in the results obtained by Arloing in Lyons and myself in Manchester.

After the Fourth Week.—Some of the lymphatic ganglia of the opposite side of the body below the diaphragm become gradually affected, but this takes place extremely slowly, and the sublumbar and popliteal glands escape for a considerable time.

It remains only for me to add that in two cases I have found sputa, which contained the tubercle bacilli in so small a number and of such characters that their presence was doubtful, give rise to a very rapid and extensive tuberculosis. In one of these cases I was able to obtain from the spleen a pure cultivation of typical tubercle bacilli three weeks after inoculation. In other cases I have found the inoculation of sputa, which teemed with bacilli, giving the most typical staining reaction, to be followed by a comparatively mild form of tuberculosis. This seems to indicate that in some cases the bacillus is either more virulent than in other cases, or that it is in certain stages of development which are not revealed by the ordinary methods of staining. In both cases it is evident that Villemin's method gives results of extreme importance. I need not point out the necessity of inoculating more than one guinea-pig in each case, though accidental mortality among guinea-pigs that are properly inoculated and well kept afterwards is extremely small during the first four weeks after inoculation.

CONCLUSIONS.

1. The inoculation method is a method of diagnosis capable of giving results free from any ambiguity.
2. The negative results obtained by it are nearly as valuable as the positive results.
3. The positive results give more definite information than the discovery of the bacillus tuberculosis.
4. Results should easily be obtained within two or three weeks.⁶

⁶ All this applies, of course, only to the ordinary forms of tuberculosis and not to certain modified forms, such as lupus, or still less to avian tuberculosis. With these forms of tuberculosis certain precautions have to be taken to obtain definite results. Bacilli which have been submitted to the action of various disinfecting agents may also act very much more slowly. I mention these exceptions, which are evident to those acquainted with the subject, in order not to mislead others by the very positive conclusions which I advance here.

